



(12) **EUROPEAN PATENT APPLICATION**

(21) Application number : **92303457.3**

(51) Int. Cl.<sup>5</sup> : **C12N 15/12, C07K 13/00,  
G01N 33/58, G01N 33/68**

(22) Date of filing : **16.04.92**

(30) Priority : **25.04.91 US 691197**  
**25.04.91 US 691200**  
**25.04.91 US 691198**  
  
 (43) Date of publication of application :  
**28.10.92 Bulletin 92/44**  
  
 (84) Designated Contracting States :  
**CH DE FR GB IT LI NL**  
  
 (71) Applicant : **MERCK & CO. INC.**  
**126, East Lincoln Avenue P.O. Box 2000**  
**Rahway New Jersey 07065-0900 (US)**

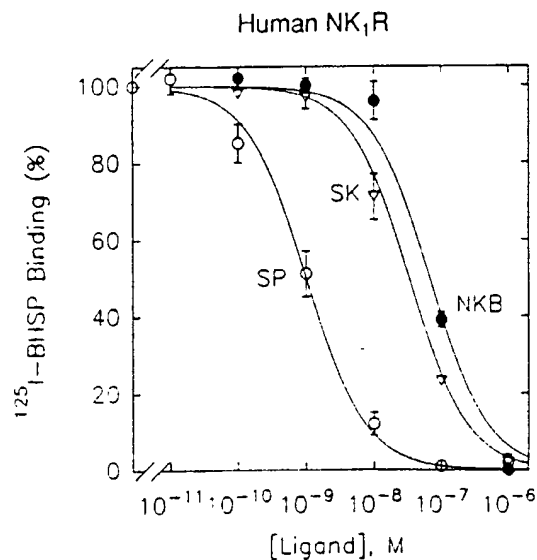
(72) Inventor : **Fong, Tung Ming**  
**38 Bolton Street**  
**Somerset, NJ 08873 (US)**  
 Inventor : **Strader, Catherine D.**  
**119 Morningside Avenue**  
**Verona, NJ 07044 (US)**  
  
 (74) Representative : **Thompson, John Dr. et al**  
**Merck & Co., Inc. European Patent**  
**Department Terlings Park Eastwick Road**  
**Harlow, Essex CM20 2QR (GB)**

(54) **Human neurokinin-1 receptor.**

(57) Recombinant human neurokinin-1 receptor (human NK1R) is disclosed which has been prepared by polymerase chain reaction techniques. Also disclosed is the complete sequence of human NK1R complementary DNA; expression systems, including a CHO (chinese hamster ovarian cell line) stable expression system; and an assay using the CHO expression system.

NK1R, also known as substance P receptor, can be used in an assay to identify and evaluate entities that bind substance P receptor. The assay can also be used in conjunction with diagnosis and therapy to determine the body fluid concentration of substance P in arthritis patients.

FIGURE 1



## BACKGROUND OF THE INVENTION

The present invention concerns cloned human neurokinin-I receptor (human NKIR) and recombinant human NKIR. Neurokinin-I receptor is also known as substance P receptor.

J. Yokota, et al., J. Biol. Chem., 264:17649 (1989) have reported cloned rat neurokinin-I receptor. N.P. Gerard, et al., J. Biol. Chem., 265:20455 (1990), have reported human neurokinin-2 receptor. Cloned rat and bovine neurokinin-2 receptor have likewise been reported. See respectively, Y. Sasi, and S. Nakanishi, Biochem Biophys. Res. Comm., 165:695 (1989), and Y. Masu, et al., Nature 329:836 (1987). Cloned rat neurokinin-3 receptor has also been reported by R. Shigemoto, et al., J. Biol. Chem., 265:623 (1990).

The above references, however, neither disclose or suggest the instant invention. In particular, the pharmacological profile of the human receptor differs significantly from the rat. Moreover, the rat neurokinin-I receptor differs from the NKIR disclosed herein by 23 amino acids.

Substance P is a naturally occurring undeca peptide belonging to the tachykinin family of peptides. Substance P is a pharmacologically-active neuropeptide that is produced in mammals. Its characteristic amino acid sequence is illustrated in U.S. 4,680,283. As is well known in the art substance P and other tachykinins have been implicated in the pathophysiology of numerous diseases. Substance P has been shown to be involved in the transmission of pain or migraine (see B.E.B. Sandberg et al., *Journal of Medicinal Chemistry*, Vol. 25, p. 1009 (1982)), as well as in central nervous system disorders such as anxiety and schizophrenia, in respiratory and inflammatory diseases such as asthma and rheumatoid arthritis, respectively, and in gastrointestinal disorders and diseases of the GI tract, like ulcerative colitis and Crohn's disease, etc. (see D. Regoli in "Trends in Cluster Headache," edited by F. Sicuteri et al., Elsevier Scientific Publishers, Amsterdam, 1987, pp. 85-95).

The instant invention also concerns an assay protocol which can be used to determine substance P activity in body fluids. The assay can also be used for identifying and evaluating substances that bind substance P receptor. Thus, the assay can be used to identify substance P antagonists and evaluate their binding affinity. Other methods includes that described by M.A. Cascieri, et al., J. Biol. Chem., 258:5158 (1983).

By use of such methods, substance P antagonists have been identified. See, for example, R. M. Snider, et al., Science, 251:435 (Jan. 1991) and S. McLean, et al., Science, 251:437 (Jan. 1991). See also WO90/05525 which published May 31, 1990, which is hereby incorporated by reference. Methods to date have proven inferior, in part, for failure of the animal receptor (animal NKIR, NK2R or NK3R) activity to accurately reflect that of human neurokinin-I receptor. Furthermore, prior to this disclosure human NKIR has not been available in a purified form or in substantial isolation from NK2R and/or NK3R.

Use of such neurokinin receptor sources can not accurately depict the affinity for human NKIR.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Full length amino acid sequence of human neurokinin-I receptor.

Figure 2 Full length nucleotide sequence of the cloned human neurokinin-I receptor complementary DNA.

Figure 3 Competitive binding of substance P (SP), substance K (SK) and human neurokinin-I receptor (NKIR) in COS assay.

## SUMMARY OF THE INVENTION

Recombinant human neurokinin-I receptor (human NKIR) is disclosed which has been prepared by polymerase chain reaction techniques. Also disclosed is the complete sequence of human NKIR complementary DNA; expression systems, including a CHO (chinese hamster ovarian cell line) stable expression system; and an assay using the CHO expression system.

NKIR, also known as substance P receptor, can be used in an assay to identify and evaluate entities that bind substance P receptor. The assay can also be used in conjunction with diagnosis and therapy to determine the body fluid concentration of substance P in arthritis patients.

## DETAILED DESCRIPTION OF THE INVENTION

One embodiment of the invention concerns human neurokinin-I receptor, said receptor being free of other human receptor proteins.

In one class this embodiment concerns human neurokinin-I receptor, said receptor being free of other human proteins.

Within this class, this embodiment concerns human neurokinin-I receptor from human cells such as glioblastoma, said receptor being free of other proteins.

In a second class, this embodiment concerns a protein comprising the 407 amino acid sequence depicted in Figure 1, said protein being free of other human receptor proteins.

Within the second class this embodiment concerns a protein consisting of the 407 amino acid sequence as shown in Figure 1.

The first embodiment also concerns a pharmaceutical composition for inhibiting the binding of substance P to cellular neurokinin-I receptor, said composition comprising an effective amount of neurokinin-I receptor.

The first embodiment also concerns a method of inhibiting the binding of substance P to cellular human neurokinin-I receptor, in a patient in need of such inhibition, comprising: administration of an effective amount of human neurokinin-I receptor.

The use of such pharmaceutical compositions and methods for antagonising the binding of substance P to in vivo neurokinin-I receptor is disclosed in, for example, R. M. Snider, et al., Science, 251:435 (Jan. 1991); S. McLean, et al., Science, 251:437 (Jan. 1991); and WO90/05525 which published May 31, 1990, which are hereby incorporated by reference.:

A second embodiment concerns a DNA sequence encoding human neurokinin receptor complementary DNA, said DNA, said sequence being free of other human DNA sequences.

As will be appreciated by those of skill in the art, there is a substantial amount of redundancy in the set of codons which translate specific amino acids. Accordingly, the invention also includes alternative base sequences wherein a codon (or codons) are replaced with another codon, such that the amino acid sequence translated by the DNA sequence remains unchanged. For purposes of this specification, a sequence bearing one or more such replaced codons will be defined as a degenerate variation. Also included are mutations (exchange of individual amino acids) which one of skill in the art would expect to have no effect on functionality, such as valine for leucine, arginine for lysine and asparagine for glutamine.

One class of the second embodiment the invention concerns the nucleotide sequence of complementary DNA, beginning with nucleotide 123 and ending with nucleotide 1346 as shown in Figure 2.

Within this class of the second embodiment is the DNA sequence that further comprises:

```

      10      20      30      40      50      60      70
GAAAAAGCCT TCCACCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GGCGCCACGA CAGGACTCTG
      80      90     100     110     120 122
CTGCAGAGGG GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG AA

```

or a degenerate variation thereof.

The second embodiment the invention concerns the partial nucleotide sequence of complementary DNA, as shown in Figure 2 or a degenerate variation thereof.

A third embodiment of this invention concerns systems for expressing human neurokinin receptor.

One class this third embodiment of the invention comprises:

A plasmid which comprises:

- (a) a mammalian expression vector, such as pRcCMV, and
- (b) a base sequence encoding human neurokinin-I receptor protein.

Within this class of the third embodiment the neurokinin-I receptor comprises the nucleotide sequence of complementary DNA, beginning with nucleotide 123 and ending with nucleotide 1346 as shown in Figure 2.

A second class of this third embodiment of the invention concerns a system for the transient expression of human neurokinin-I receptor (NKIR) in a monkey kidney cell line (COS).

A third class of this third embodiment of the invention concerns a system for the expression of human neurokinin-I receptor in a chinese hamster ovarian cell line (CHO), the system comprising a vector comprising human neurokinin receptor (NKIR) cDNA.

Within this class of the third embodiment is the sub-class wherein the expression system includes

A plasmid which comprises:

- (a) a mammalian expression vector, such as pRcCMV, and
- (b) a base sequence encoding human neurokinin-I receptor protein.

Within this sub-class the neurokinin-I receptor comprises the nucleotide sequence of complementary DNA, beginning with nucleotide 123 and ending with nucleotide 1346 as shown in Figure 2, is subclosed into the vector pRcCMV.

A forth embodiment of the invention concerns a method of using any of the above expression systems for determining the binding affinity of a test sample for human neurokinin-I receptor.

In one class this embodiment concerns a method of using a Chinese hamster ovarian cell line, said line

transplanted with a plasmid, which plasmid comprises:

(a) vector pRcCMV, and

(b) the base sequence encoding human neurokinin-I receptor protein, the method which comprises:

(1) expressing human neurokinin-I receptor in said CHO cells;

(2) addition of a test sample to a solution containing  $^{125}\text{I}$ -substance P and said cells;

(3) incubating the products of Step (1), wherein said incubation effective for expressing said the human neurokinin-I receptor and effective for competitive binding of said  $^{125}\text{I}$ -substance P and said test sample to said human neurokinin-I receptor;

(4) separating said  $^{125}\text{I}$ -substance P which is bound to said human neurokinin-I receptor from said  $^{125}\text{I}$ -substance P which is not bound;

(5) measuring the radioactivity of said  $^{125}\text{I}$ -substance P which is bound to said human neurokinin-I receptor.

In a second class this embodiment concerns a method of using a Chinese hamster ovarian cell line (CHO), said line transplanted with a plasmid which plasmid comprises

(a) vector pRcCMV, and

(b) the base sequence encoding human neurokinin-I receptor protein, the method comprising:

(1) expressing human neurokinin-I receptor in said CHO cells;

(2) equilibrating the product of Step (1) with  $^3\text{H}$ -myo-inositol;

(3) washing the product of Step (2);

(4) incubating the product of Step (3) with a test sample in the presence of 10 mM LiCl, which results in the production of inositol monophosphate;

(5) measuring the inositol monophosphate.

In overview, the present invention describes methods to isolate the human neurokinin-I receptor (human NKIR) complementary DNA (cDNA) without prior knowledge of its protein sequence or gene sequence. Human NKIR is a membrane receptor for the neurotransmitter substance P. Polymerase chain reaction (PCR) technique was utilized for the isolation of human NKIR cDNA. In the approach, the regions of rat NKIR Applicants thought to be similar to human NKIR were identified, oligonucleotide primers corresponding to those region were designed, PCR amplification was carried out to obtain part of the NKIR cDNA from human cells, and its DNA sequence was determined. The remaining part of the human NKIR cDNA was obtained from a human cDNA library utilizing the above sequence information of human NKIR cDNA.

The complete sequence of the human NKIR cDNA was determined, and its encoded protein sequence was deduced. Among other things, such sequence information is useful in the process of developing novel substance P antagonists.

Three heterologous expression systems were used to express the cloned human NKIR cDNA. The Xenopus oocyte expression enables one to determine the biological function of human NKIR. The COS (a monkey kidney cell line) expression can be used to measure the ligand binding properties of human NKIR. The CHO (a Chinese hamster ovarian cell line) stable expression is suitable for natural product screen to identify potential therapeutic agent or other substances that bind to substance P receptor. This cell line can also be used as an assay kit for determining the body fluid concentration of substance P in arthritis patients.

Assay protocols use the heterologously expressed human NKIR for determination of the binding affinity and antagonistic activity of substance P antagonists.

#### 1) Isolation of human NKIR cDNA

To isolate the human NKIR cDNA in the absence of its sequence information, we developed methods to obtain three separate but overlapping cDNA clones in three steps. (i) We have adopted the homologous cloning strategy (Ohara et al., 1989, Proc. Nat. Acad. Sci., 86:5673-5677) to isolate cDNA clones encoding the central core region of human NKIR, with the assumption that the human NKIR sequence is similar to the published sequence (Yokota et al., 1989, J. Biol. Chem., 264:17649-17652) of rat NKIR in certain areas where appropriate PCR primers can be designed. Degenerate primers corresponding to the rat sequence were used in PCR amplification (Mullis and Faloona, 1987, Meth. Enzymol., 155:335) to obtain the cDNA encoding the central transmembrane core region of human NKIR from human mRNA. (ii) After determining the sequence of the core region in human NKIR, new primers corresponding to the human sequence were designed and a second homologous PCR amplification was performed using the human primer in the core region with degenerate primers corresponding to the N-terminal sequence of rat NKIR. The cDNA encoding the N-terminal region of human NKIR was thus obtained from human mRNA and its sequence was determined. (iii) An anchored PCR strategy was developed to isolate the cDNA encoding the C-terminal region of human NKIR, in which primers corresponding to the core region of human NKIR were used in combination with a primer corresponding to the se-

quence of a cloning vector to obtain the cDNA from a human cDNA library.

To confirm the authenticity of the cDNA encoding human NKIR, an independent PCR amplification was performed to obtain the full length cDNA in a single step using primers from the 5' and 3' untranslated regions.

## 5 2) Expression of the cloned human NKIR

Three expression systems were developed for the cloned human NKIR. An transient expression in *Xenopus* oocytes resulted from microinjection of in vitro transcribed mRNA from the cloned cDNA (*Xenopus* Laevis from XENOPUS ONE, Ann Arbor, MI). This system allows the measurement of biological effect of NKIR activation upon ligand binding. Another transient expression in COS (a monkey kidney cell line, ATCC CRL 1651, ATCC Rockville MD) resulted from the transfection of the cloned cDNA under the control of viral promoter into mam-  
 10 malian cells (e.g., COS). The transfected cells are suitable for determination binding affinity of human NKIR for various ligands. Stable expression of human NKIR in mammalian cells (e.g., CHO, a Chinese hamster ovarian cell line, ATCC CRL 9096, ATCC Rockville MD) was achieved after integration of the transfected cDNA  
 15 into the chromosomes of the host cells. These stable cell lines will constitutently express the cloned human NKIR and can be propagated infinitely. Therefore, stable expression system is very useful in large scale drug screen, and can be used to determine substance p concentration in the biopsy sample of patients.

To establish a stable cell line expressing the cloned human NKIR, the cDNA was subcloned into the vector pRcCMV (INVITROGEN).

20 The electrophysiological assay of human NKIR expressed in *Xenopus* oocytes was based on the fact that NKIR activates the phospholipase C upon substance P binding, and phospholipase C in turn increases the intracellular calcium concentration through inositol trisphosphate ( $IP_3$ ) and  $IP_3$ -gated calcium channel on intracellular membranes. The calcium increase activates calcium-gated chloride channels on plasma membranes which gives rise to a chloride current measurable by two electrode voltage clamp.

25 The binding assay of human NKIR expressed in COS or CHO is based on the use of  $^{125}I$ -substance P ( $^{125}I$ -SP, from DU PONT, Boston, MA) as a radioactively labeled ligand which compete with unlabeled substance p or any other ligand for binding to the human NKIR. Monolayer cell culture of COS or CHO was dissociated by the non-enzymatic solution (SPECIALTY MEDIA, Lavallete, NJ) and resuspended in appropriate volume of the binding buffer (50 mM Tris pH 7.5, 5 mM  $MnCl_2$ , 150 mM NaCl, 0.04 mg/ml bacitracin, 0.004 mg/ml leupeptin,  
 30 0.2 mg/ml BSA, 0.01 mM phosphoramidon) such that 200  $\mu$ l of the cell suspension would give rise to about 10,000 cpm of specific  $^{125}I$ -SP binding (approximately 50,000 to 200,000 cells).

The activation of phospholipase C by NKIR can also be measured in CHO cells by determining the accumulation of inositol monophosphate which is a degradation product of  $IP_3$ .

In addition to large scale drug screening using the stable CHO cell line expressing the cloned human NKIR,  
 35 other alternative applications are obvious. For example, the stable cell line can be used in the binding assay to determine the substance p concentration from biopsy samples. The human NKIR protein can also be injected into patients to reduce substance P concentration in some neurogenic inflammatory diseases.

## EXAMPLE I

### 40 Step A:

In the first step of obtaining the cDNA encoding the central core region of human NKIR, human mRNA was prepared from three human glioblastoma cell lines T98G, CCF-STTG1 and U87MG (obtained from the American  
 45 Type Culture Collection, Rockville, MD) by the FASTTRACK method (INVITROGEN, San Diego, CA). Synthesis of first strand cDNA from 4  $\mu$ g of human mRNA was initiated by oligo (dT) primers in a total volume of 20  $\mu$ l according to protocols of the BRL cDNA synthesis system (BRL, LIFE TECHNOLOGIES, Inc., Gaithersburg, MD). Ten  $\mu$ l of the first strand cDNA was used as template with three rat primers (50 pmol rspr2s4, 50 pmol rspr2s4h, and 100 pmol rspr7a2; see Table I for their sequences) in a primary PCR amplification in a total volume  
 50 of 100  $\mu$ l according to the GENEAMP protocol (PERKIN ELMER CETUS, Norwalk, CT). Thirty cycles of PCR were performed using the following parameters: 1 min of denaturation at 94°C, 2 min of annealing at 40°C and 4 min of extension at 72°C with 2 sec of auto extension. Ten  $\mu$ l of the primary PCR product was used as template with the same primers in a secondary PCR amplification under the same cycling conditions to further amplify the DNA. Ten  $\mu$ l of the secondary PCR product was used as template with three rat primers (50 pmol rspr2s4,  
 55 50 pmol rspr2s4h, 50 pmol rspr7a1 and 50 pmol rspr7alh) in 30 cycles of tertiary PCR amplification with the following parameters: 1 min of denaturation at 94°C, 2 min of annealing at 45°C, and 4 min of extension at 72°C with 2 sec of auto extension. The tertiary PCR product was analyzed by agarose gel electrophoresis and was found to contain a 600 bp DNA fragment. This DNA fragment was excised from the gel, purified by GENECLAN

(Bio lOl, La Jolla, CA), phosphorylated, and subcloned into Sma I site of the plasmid vector BLUESCRIPT SK+ (STRATAGENE, La Jolla, CA). The DNA sequence was determined by the Sequenase dideoxy chain termination method (USBC, Cleveland, OH). Sequence alignment analysis showed that this cDNA fragment is similar (90% identity at nucleotide level) to the central core region of rat NKIR from amino acid 91 to 280.

#### STEP B:

After determination of the core region sequence of human NKIR, five antisense primers were synthesized based on the human sequence (hspr3a5, hspr5a1, hspr5a2, hspr6a1 and hspr6a2; see Table II for their sequences). These primers would be used to obtain the N-terminal cDNA sequence of human NKIR. One ug of human glioblastoma mRNA and 6 uM of each of the above primers was used in first strand cDNA synthesis in a total volume of 20 ul according the BRL cDNA synthesis protocols. The cDNA was extracted by phenol-chloroform, precipitated by ethanol and dissolved in 30 ul of water. Ten ul of the cDNA was used as template with two rat primers (50 pmol rsprn and 50 pmol rsprnh) and one human primer (150 pmol hspr3a5) in the primary PCR amplification in a total volume of 100 ul. Thirty cycles were performed with the following parameters: 1 min denaturation at 94°C, 1 min of annealing at 55°C, and 3 min of extension at 72°C. Five ul of the primary PCR product was then used as template with two rat primers (50 pmol rsprn and 50 pmol rsprnh) and one human primer (100 pmol hspr3a4) in 30 cycles of secondary PCR amplification with the same parameters. Two ul of the secondary PCR product was used as template with two rat primers (50 pmol rsprn and 50 pmol rsprnh) and one human primer in 30 cycles of tertiary PCR amplification with the same parameters. The tertiary PCR product was analyzed by agarose gel electrophoresis and was found to contain a 500 bp fragment. This DNA fragment can hybridize with a human oligonucleotide (hspr3a2), indicating it is not a non-specific by-product. This DNA fragment was excised from the gel, purified by GENECLEAN (Bio lOl), phosphorylated, and subcloned into Sma I site of the vector Bluescript SK+. DNA sequence analysis revealed that this fragment encodes the human NKIR N-terminal region and it also contains 5' untranslated sequence.

#### STEP C:

In the third step, an anchored PCR protocol was developed in which the cDNA encoding the C-terminal region of human NKIR was obtained from a cDNA library using sense human primers and a primer corresponding to the vector sequence. Three ug of human glioblastoma mRNA was primed by 2.5 ug of oligo (dT) in the first strand cDNA synthesis in a total volume of 50 ul, followed by second strand cDNA synthesis according the BRL cDNA synthesis protocols. The cDNA product was then heated at 70°C for 10 min. The yield of double stranded cDNA was determined by incorporating 1.25 uM of <sup>32</sup>P- $\alpha$ -dCTP as tracer in the reaction. Four ul of T4 DNA polymerase was added to the reaction mixture and incubated at 37°C for 10 min. The reaction was stopped by adding 16 ul of 250 mM EDTA, extracting with phenol/CHCl<sub>3</sub>, and precipitating with ethanol. The cDNA was dissolved in 50 ul of HE buffer (10 mM HEPES-1mM EDTA). Small size cDNA was removed by the Select-D(RF) SPIN COLUMN (5'TO3', Boulder, CO), and the large size cDNA was precipitated by ethanol and dissolved in 36 ul of water. Four ul of 0.2 M Tris-10 mM spermidine-1 mM EDTA (pH7.5) was added to the tube and heated at 70°C for 1 min. The cDNA was phosphorylated by adding 5 ul of blunt-end kinase buffer (0.5 M Tris pH 9.5, 0.1 M MgCl<sub>2</sub>, 50 mM DTT, 50% glycerol), 2.5 ul of 10 mM ATP, 2.5 ul of polynucleotide kinase, and incubating at 37°C for 30 min. The cDNA was extracted by phenol/CHCl<sub>3</sub>, precipitated by ethanol and ligated to EcoRI linker according to the PROMEGA ECOR1 linker ligation protocol (PROMEGA, Madison, WI). Linker-ligated cDNA was then ligated to calf intestinal phosphatase-treated EcoRI site of the vector BLUESCRIPT SK+. One ul of the ligated plasmid DNA was used as template in 30 cycles of primary PCR with two human primers (50 pmol hspr6s1 and 50 pmol hspr6s2) and 100 pmol of vector-specific primer t3 (obtained from STRATAGENE) with the following parameters: 1 min of denaturation at 94°C, 2 min of annealing at 55°C, and 4 min of extension at 72°C with 2 sec auto extension. One ul of the primary PCR product was used in 30 cycles of secondary PCR amplification with one human primer (100 pmol hspr6s3) and the same vector-specific primer t3 under the same conditions. One ul of the secondary PCR product was used in 30 cycles of tertiary PCR amplification with one human primer (100 pmol hspr6s4) and 100 pmol of vector-specific primer SK (STRATAGENE) under the same conditions. A 780 bp DNA fragment was detected which also hybridized to a human oligo probe hspr6s5. This DNA fragment was excised from the agarose gel, purified by GENECLEAN (BIO lOl), phosphorylated, and subcloned into Sma I site of the vector BLUESCRIPT SK+. DNA sequence analysis revealed that it encodes the C-terminal region of human NKIR and contains 3' untranslated sequence.

**STEP D:**

Since three separate but overlapping cDNA clones encoding human NKIR were isolated above and the possibility of alternative pre-mRNA splicing exists, it is necessary to confirm the authenticity of the full length cDNA sequence by isolating a full length cDNA directly. Based on the above sequence in the untranslated region, primers were synthesized which should give rise to a full length cDNA. Using the PERKIN ELMER CETUS RNA PCR amplification kit (Perkin Elmer Cetus), cDNA was synthesized from 1.5 ug of human glioblastoma mRNA in a total volume of 20 ul with 50 pmol of the human primer hspr3uta5. One half of the first strand cDNA was used as template in 30 cycles of primary PCR amplification with two human primers (50 pmol hspr3uta5, 50 pmol hspr5uts1) with the following parameters: 1 min of denaturation at 94°C, 2 min of annealing at 55°C, and 4 min of extension at 55°C with 2 sec auto extension. Ten ul of the primary PCR product was used as template in 30 cycles of secondary PCR amplification with two human primers (50 pmol hspr3uta6 and 50 pmol hspr5uts2) under the same conditions. A 1350 bp DNA fragment was excised from agarose gel, purified by GEN-ECLEAN (BIO 101), digested with restriction endonucleases with EcoRI and Not I, and subcloned into the vector BLUESCRIPT SK+. DNA sequence analysis confirmed the general structure of the cloned human NKIR cDNA. The sequence of human NKIR cDNA is shown in Figure I.

Table I: Primers based on rat NKIR sequence. The last letter "h" in some primers denotes that human codon bias was incorporated (Lathe, 1985, J. Mol. Biol., 183:1-12). The position number in the rat cDNA sequence was defined by Yokota et al. (J. Biol Chem., 1989, 264:17649-17652).

Name	Sequence	Position	Direction
rspr2s4	tgcatggctgcattcaat	238 - 255	sense
rspr2s4h	tgcatggctgccttcaa	238 - 254	sense
rspr7a2	acagtagatgatggggtgtacat	918 - 894	antisense
rspr7a1	caggtagacctgctggatgaactt	864 - 841	antisense
rspr7alh	caggtacacctgctggatgaactt	864 - 841	antisense
rsprn	atggataacgtccttccctat	1 - 20	sense
rsprnh	atggacaatgtgctgccca	1 - 19	sense

Table II: Primers based on the human NKIR cDNA sequence. Position number is defined in the sequence listing in the text. The nucleotides in parentheses are not present in the human NKIR cDNA; they are restriction sites for subcloning purpose.

	Name	Sequence	Position	Direction
5	hspr3a2	gaagaagttgtggaacttgca	455 - 435	antisense
	hspr3a1	catggagtagatactggcgaa	491 - 471	antisense
	hspr3a4	ggatgtatgatggccatgta	532 - 513	antisense
	hspr3a5	actttggtggctgtggctga	568 - 549	antisense
	hspr5a1	atgcatagccaatcaccagca	768 - 748	antisense
10	hspr5a2	catagtgtgattcccactac	793 - 774	antisense
	hspr6a1	tgcacaccacgacaatcatca	888 - 868	antisense
	hspr6a2	ttgatgtagggcaggaggaa	943 - 924	antisense
	hspr6s1	gcaagtctctgccaagcgcaa	836 - 856	sense
	hspr6s2	tgatgattgtcgtggtgtgca	868 - 888	sense
15	hspr6s3	ttccacatcttcttctcct	912 - 931	sense
	hspr6s4	ctacatcaaccagatctct	935 - 954	sense
	hspr6s5	tctctacctgaagaagtt	950 - 967	sense
	hspr3uta5	caaggatggaatgttttccct	1499 - 1479	antisense
	hspr3uta6	(gacatgcggccgc)aaccatactgaccctttt	1478 - 1460	antisense
20	hspr5uts1	cctcctgtctggcttttagaa	16 - 35	sense
	hspr5uts2	(gcgcagaattc)gtgtacagatagtaggctt	86 - 105	sense

#### Expression in Xenopus oocytes

25 To express the human NKIR cDNA in *Xenopus* oocytes, the cDNA was cloned into an *in vitro* transcription vector BLUESCRIPT SK+ (STRATAGENE) which contains the T7 promoter for initiation of T7 RNA polymerase catalyzed RNA synthesis. One ug of linear plasmid DNA which contained the human NKIR cDNA downstream of the T7 promoter was used in the *in vitro* transcription reaction containing 40 mM Tris pH 7.5, 50 mM NaCl, 8 mM MgCl<sub>2</sub>, 2 mM spermidine, 0.4 mM CTP, 0.4 mM ATP, 0.4 mM UTP, 0.16 mM GTP, 2.5 ul CAP analog (STRATAGENE), 30 mM DTT, 1 U RNase Block II (STRATAGENE), 0.83 pmol <sup>32</sup>P- $\alpha$ -CTP and 25 U of T7 RNA polymerase. The reaction tube was incubated at 37°C for 1 hour. Usually 5 ug of RNA was synthesized as quantitated by incorporation of <sup>32</sup>P- $\alpha$ -CTP into RNA. After RNA synthesis, the plasmid DNA was removed by adding 10 U of RNase free DNase and 1 U of RNase Block II. The reaction mixture was extracted by phenol/CHCl<sub>3</sub>, and the unincorporated nucleotides were removed by the Select-D(RF) spin column (5'TO3'). The RNA transcript 35 was precipitated by ethanol twice and dissolved in RNase free water. Oocytes were removed from *Xenopus* frogs, treated with 2 mg/ml collagenase (specific activity < 0.3 U/mg, BOEHRINGER MANNHEIM, Indianapolis, IN) in OR-2 buffer (82.5 mM NaCl, 2 mM KCl, 1 mM MgCl<sub>2</sub>, 5 mM HEPES, pH 7.4) for 4 hours at 19°C. The dissociated oocytes were incubated in OR-2 buffer supplemented by 1.8 mM CaCl<sub>2</sub>, 0.5 mg/ml gentamycin and 0.5 mM theophylline at 19°C overnight before injection. A 50 nl aliquot contain 2 ng of RNA transcript was injected into each oocyte. The injected oocytes were incubated at 19°C for 2 days before electrophysiological recording (see Example 3 for assay method).

#### Expression in COS

45 To express the human NKIR transiently in COS, the cDNA was cloned into the expression vector pCDM9 which was derived from pCDM8 (INVITROGEN) by inserting the ampicillin resistance gene (nucleotide 1973 to 2964 from BLUESCRIPT SK+) into the Sac II site. Transfection of 20 ug of the plasmid DNA into 10 millions COS cells was achieved by electroporation in 800 ul of transfection buffer (135 mM NaCl, 1.2 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 2.4 mM K<sub>2</sub>HPO<sub>4</sub>, 0.6 mM KH<sub>2</sub>PO<sub>4</sub>, 10 mM glucose, 10 mM HEPES pH 7.4) at 260 V and 950 uF using the IBI GENEZAPPER (IBI, New Haven, CT). The cells were incubated in 10% fetal calf serum, 2 mM glutamine, 100U/ml penicillin-streptomycin, and 90% DMEM media (GIBCO, Grand Island, NY) in 5% CO<sub>2</sub> at 37°C for three days before the binding assay.

#### Stable Expression in CHO

55 To establish a stable cell line expressing the cloned human NKIR, the cDNA was subcloned into the vector pRcCMV (INVITROGEN). Transfection of 20 ug of the plasmid DNA into CHO cells was achieved by electroporation in 800 ul of transfection buffer supplemented with 0.625 mg/ml Herring sperm DNA at 300 V and 950



uF using the IBI GENEZAPPER (IBI). The transfected cells were incubated in CHO media [10 % fetal calf serum, 100 U/ml penicillin-streptomycin, 2 mM glutamine, 1/500 hypoxanthine-thymidine (ATCC), 90% IMDM media (JRH BIOSCIENCES, Lenexa, KS), 0.7 mg/ml G418 (GIBCO)] in 5% CO<sub>2</sub> at 37°C until colonies were visible. Each colony was separated and propagated. The cell clone with the highest number of human NKIR was selected for subsequent application in the assay of Example 3.

### Example 3

#### Assay Protocol Using Oocytes

The oocyte was voltage-clamped at - 80 mV by the model 8500 intracellular preamp-clamp (DAGAN, Minneapolis, MN). The recording chamber was continuously perfused with recording buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl<sub>2</sub>, 5 mM HEPES, pH 7.4). Chloride current was elicited by applying substance P (from 0.1 nM to 1000 nM) to the recording chamber. At least three oocytes were measured for each concentration. The antagonistic activity of any potential substance P antagonist can be assessed by determining the inhibition of substance P response. Likewise, NK1 agonists can be identified by their ability to stimulate a response in oocytes injected with NKIR mRNA but not in uninjected oocytes.

#### Assay Protocol using COS or CHO

The binding assay of human NKIR expressed in COS or CHO is based on the use of <sup>125</sup>I-substance P (<sup>125</sup>I-SP, from DU PONT, Boston, MA) as a radioactively labeled ligand which compete with unlabeled substance p or any other ligand for binding to the human NKIR. Monolayer cell culture of COS or CHO was dissociated by the non-enzymatic solution (SPECIALTY MEDIA, Lavallette, NJ) and resuspended in appropriate volume of the binding buffer (50 mM Tris pH 7.5, 5 mM MnCl<sub>2</sub>, 150 mM NaCl, 0.04 mg/ml bacitracin, 0.004 mg/ml leupeptin, 0.2 mg/ml BSA, 0.01 mM phosphoramidon) such that 200 ul of the cell suspension would give rise to about 10,000 cpm of specific <sup>125</sup>I-SP binding (approximately 50,000 to 200,000 cells). In the binding assay, 200 ul of cells were added to a tube containing 20 ul of 1.5 to 2.5 nM of <sup>125</sup>I-SP and 20 ul of unlabeled substance p or any other test compound. The tubes were incubated at 4°C or at room temperature for 1 hour with gentle shaking. The bound radioactivity was separated from unbound radioactivity by GF/C filter (BRANDEL, Gaithersburg, MD) which was pre-wetted with 0.1 % polyethylenimine. The filter was washed with 3 ml of wash buffer (50 mM Tris pH 7.5, 5 mM MnCl<sub>2</sub>, 150 mM NaCl) three times and its radioactivity was determined by gamma counter. Illustrative of this method of using these expression systems are the results shown in Figure 3. These results show the competitive binding of substance P (SP), substance K (SK) and human neurokinin-1 receptor (NKIR) in the COS assay.

#### ALTERNATIVE PROTOCOL

The activation of phospholipase C by NKLR can also be measured in CHO cells by determining the accumulation of inositol monophosphate which is a degradation product of IP<sub>3</sub>. CHO cells were seeded in 12-well plate at 250,000 cells per well. After incubating in CHO media for 4 days, cells were loaded with 0.025 uCi/ml of <sup>3</sup>H-myoinositol by overnight incubation. The extracellular radioactivity was removed by washing with phosphate buffered saline. LiCl was added to the well at final concentration of 0.1 mM with or without antagonist, and continued incubation at 37°C for 15 min. Substance p was added to the well at final concentration of 0.3 nM to activate the human NKIR. After 30 min of incubation at 37°C, the media was removed and 0.1 N HCl was added. Each well was sonicated at 4°C and extracted with CHCl<sub>3</sub>/methanol (1:1). The aqueous phase was applied to a 1 ml Dowex AG IX8 ion exchange column. The column was washed with 0.1 N formic acid followed by 0.025 M ammonium formate-0.1 N formic acid. The inositol monophosphate was eluted with 0.2 M ammonium formate-0.1 N formic acid and quantitated by beta counter.

SEQUENCE LISTING

5

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 122 base pairs

(B) TYPE: nucleic acid

10

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

15

20

25

30

35

40

45

50

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAAAAAGCCT TCCACCCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA 50  
 5 GGCGCCACGA CAGGACTCTG CTGCAGAGGG GGGTTGTGTA CAGATAGTAG 100  
 GGCTTTACCG CCTAGCTTCG AA 122

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 18 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGCATGGCTG CATTCAAT 18

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 17 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGCATGGCTG CCTCAA 17

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACAGTAGATG ATGGGGTTGT ACAT

24

5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE:

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGGTAGACC TGCTGGATGA ACTT

24

20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE:

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAGGTAGACC TGCTGGATGA ACTT

24

35

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE:

45

50

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGATAACG TCCTTCCTAT

20

5

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE:

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGGACAATG TGCTGCCCA

19

20

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE:

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAAGAAGTTG TGGAACTTGC A

21

35

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE:

45

50

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATGGAGTAG ATACTGGCGA A

21

5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE:

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGATGTATGA TGGCCATGTA

20

20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE:

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACTTGGTGG CTGTGGCTGA

20

35

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE:

45

50

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGCATAGCC AATCACCAGC A

21

5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE:

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CATAGTGTGA TTCCCACTAC

20

20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE:

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGCACACCAC GACAATCATC A

21

35

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE:

45

50

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTGATGTAGG GCAGGAGGAA

20

5

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE:

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCAAGTCTCT GCCAAGCGCA A

21

20

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE:

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TGATGATGT CGTGGTGTGC A

21

35

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE:

45

50

55



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTCCACATCT TCTTCCTCCT

20

5

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE:

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTACATCAAC CCAGATCTCT

20

20

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE:

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCCTACCTG AAGAAGTT

18

35

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE:

45

50

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CAAGGATGGA ATGTTTTCCC T

21

5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE:

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GACATGCGGC CGCAACCCAT ACTGACCCCT TT

32

20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE:

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CCTCCTGTCT GGCTTAGAA

20

35

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE:

45

50

55

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCGCAGAATT CGTGACAGA TAGTAGGCTT

30

5

## (2) INFORMATION FOR SEQ ID NO:26:

## (i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH: 1224 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

## (ii) MOLECULE TYPE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

20

ATGGATAA CGTCCTCCCG 140

GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT CGGAACCCAA TCAGTTCGTG 200

25

CAACCAGCCT GGCAAATGT CTTTGGGCA GCTGCCTACA CGGTATTGT GGTGACCTCT 260

GTGGTGGGCA ACGTGGTAGT GATGTGGATC ATCTTAGCCC ACAAAGAAT GAGGACAGTG 320

ACGAACTATT TTCTGGTGAA CCTGGCCCTC GCGGAGGCT CCATGGCTGC ATTCAATACA 380

30

GTGGTGAAC TACCTATGC TGTCCACAAC GAATGGTACT ACGGCTGTT CTACTGCAAG 440

TTCCACAAC TCTCCCAT CGCCGTGTC TTCGCCAGTA TCTACTCCAT GACGGCTGTG 500

35

GCCCTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC CCGGCTGTC AGCCACAGCC 560

ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC TGTGGCCTT CCCCAGGGC 620

TACTACTCAA CCACAGAGAC CATGCCAGC AGAGTGTGT GCATGATCGA ATGGCCAGAG 680

40

CATCCGAACA AGATTATGA GAAAGTGAC CACATCTGTG TGACTGTGCT GATCTACTTC 740

CTCCCCCTGC TGGTGATTGG CTATGCATAC ACCGAGTGG GAATCAGACT ATGGGCCAGT 800

GAGATCCCG GGGACTCTC TGACCGCTAC CACGAGCAAG TCTCTGCCAA GCGCAAGGTG 860

45

GTCAAAATGA TGATTGTGCT GGTGTGCACC TTCGCCATCT GCTGGCTGCC CTTCACATC 920

TTCTTCTCC TGCCCTACAT CAACCCAGAT CTCTACCTGA AGAAGTTAT CCAGCAGGTC 980

50

TACCTGGCCA TCATGTGGCT GGCCATGAGC TCCACCATGT ACAACCCCAT CATCTACTGC 1040

55

TGCCTCAATG ACAGGTTCCG TCTGGGCTTC AAGCATGCCT TCCGGTGCTG CCCCTTCATC 1100  
 AGCGCCGGCG ACTATGAGGG GCTGGAATG AAATCCACCC GGTATCTCCA GACCCAGGGC 1160  
 5 AGTGTGTACA AAGTCAGCCG CCTGGAGACC ACCATCTCCA CAGTGGTGGG GGCCCACGAG 1220  
 GAGGAGCCAG AGGACGGCCC CAAGGCCACA CCTCGTCCC TGGACCTGAC CTCCAAGTGC 1280  
 10 TCTTCACGAA GTGACTCAA GACCATGACA GAGAGCTTCA GCTTCTCCTC CAATGTGCTC 1340  
 TCCTAG 1346

## (2) INFORMATION FOR SEQ ID NO:27:

15

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 20 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE:

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GCCA CAGGGCCTTT GGCAGGTGCA GCCCCACTG CCTTTGACCT 1390  
 GCCTCCCTTC ATGCATGGAA ATTCCCTTCT TCTGGAACCA TCAGAAACAC CCTCAGACTG 1450  
 30 GGACTTGCAA AAAGGGTCAG TATGGGTTAG GGAAAACATT CCATCCTTGA GTCAAAAAAT 1510  
 CTCAATTCTT CCCATCTTT GCCACCTTCA TGCTGTGTGA CTCAAACCAA ATCACTGAAC 1570  
 35 TTGCTGAGC CTGTAAAATA AAAGGTGGGA CCAGCTTTTC CCAAAAGCCC ATTCATTCCA 1630  
 TTCTGGAAGT GACTTTGGCT GCATGCGAGT GGTCAATTCA GGATGAATT 1679

40

## (2) INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1346 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 45 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE:

50

55

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

	GAAAAAGCCT TCCACCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GGCGCCACGA	60
5	CAGGACTCTG CTGCAGAGGG GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG	120
	AAATGGATAA CGTCCTCCCG GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT	180
10	CGGAACCCAA TCAGTTCGTG CAACCAGCCT GGCAAATTGT CCTTTGGGCA GCTGCCTACA	240
	CGGTCAATTGT GGTGACCTCT GTGGTGGGCA ACGTGGTAGT GATGTGGATC ATCTTAGCCC	300
	ACAAAAGAAT GAGGACAGTG ACGAACTATT TTCTGGTGAA CCTGGCCTTC GCGGAGGCCT	360
15	CCATGGCTGC ATTCAATACA GTGGTGAAC TACCTATGC TGCCACAAC GAATGGTACT	420
	ACGGCCTGTT CTACTGCAAG TTCCACAAC TCTCCCCAT CGCCGCTGTC TTCGCCAGTA	480
20	TCTACTCCAT GACGGCTGTG GCCTTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC	540
	CCCGGCTGTC AGCCACAGCC ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC	600
	TGCTGGCCTT CCCCCAGGGC TACTACTCAA CCACAGAGAC CATGCCCAGC AGAGTCGTGT	660
25	GCATGATCGA ATGGCCAGAG CATCCGAACA AGATTATGA GAAAGTGAC CACATCTGTG	720
	TGACTGTGCT GATCTACTTC CTCCCCCTGC TGGTGATTGG CTATGCATAC ACCGTAGTGG	780
30	GAATCACACT ATGGGCCAGT GAGATCCCCG GGGACTCCTC TGACCGCTAC CACGAGCAAG	840
	TCTCTGCCAA GCGCAAGGTG GTCAAAATGA TGATTGTCTT GGTGTGCACC TTCGCCATCT	900
	GCTGGCTGCC CTCCACATC TTCTTCTCC TGCCCTATAT CAACCCAGAT CTCTACCTGA	960
35	AGAAGTTTAT CCAGCAGGTC TACCTGGCCA TCATGTGGCT GGCATGAGC TCCACCATGT	1020
	ACAACCCCAT CATCTACIGC TGCTTCAATG ACAGGTTCGG TCTGGGCTTC AAGCATGCCT	1080
40	TCCGGTGCTG CCCCTTCATC AGCGCCGGCG ACTATGAGGG GCTGGAAATG AAATCCACCC	1140
	GGTATCTCCA GACCCAGGGC AGTGTGTACA AAGTCAGCG CCTGGAGACC ACCATCTCCA	1200
	CAGTGGTGGG GGCCACGAG GAGGAGCCAG AGGACGGCCC CAAGGCCACA CCTCGTCCC	1260
45	TGGACCTGAC CTCCAACGTC TCTTACGAA GTGACTCCAA GACCATGACA GAGAGCTTCA	1320
	GCTTCTCTC CAATGTGCTC TCCTAG	1346

50

55

## (2) INFORMATION FOR SEQ ID NO:29:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 407 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Met Asp Asn Val Leu Pro Val Asp Ser Asp Leu Ser Pro Asn Ile Ser  
 1 5 10 15

Thr Asn Thr Ser Glu Pro Asn Gln Phe Val Gln Pro Ala Trp Gln Ile  
 20 25 30

Val Leu Trp Ala Ala Ala Tyr Thr Val Ile Val Val Thr Ser Val Val  
 35 40 45

Gly Asn Val Val Val Met Trp Ile Ile Leu Ala His Lys Arg Met Arg  
 50 55 60

Thr Val Thr Asn Tyr Phe Leu Val Asn Leu Ala Phe Ala Glu Ala Ser  
 65 70 75 80

Met Ala Ala Phe Asn Thr Val Val Asn Phe Thr Tyr Ala Val His Asn  
 85 90 95

Glu Trp Tyr Tyr Gly Leu Phe Tyr Cys Lys Phe His Asn Phe Phe Pro  
 100 105 110

Ile Ala Ala Val Phe Ala Ser Ile Tyr Ser Met Thr Ala Val Ala Phe  
 115 120 125

Asp Arg Tyr Met Ala Ile Ile His Pro Leu Gln Pro Arg Leu Ser Ala  
 130 135 140

Thr Ala Thr Lys Val Val Ile Cys Val Ile Trp Val Leu Ala Leu Leu  
 145 150 155 160

Leu Ala Phe Pro Gln Gly Tyr Tyr Ser Thr Thr Glu Thr Met Pro Ser  
 165 170 175

Arg Val Val Cys Met Ile Glu Trp Pro Glu His Pro Asn Lys Ile Tyr  
 180 185 190

Glu Lys Val Tyr His Ile Cys Val Thr Val Leu Ile Tyr Phe Leu Pro  
 195 200 205  
 5 Leu Leu Val Ile Gly Tyr Ala Tyr Thr Val Val Gly Ile Thr Leu Trp  
 210 215 220  
 Ala Ser Glu Ile Pro Gly Asp Ser Ser Asp Arg Tyr His Glu Gln Val  
 225 230 235 240  
 10 Ser Ala Lys Arg Lys Val Val Lys Met Met Ile Val Val Val Cys Thr  
 245 250 255  
 Phe Ala Ile Cys Trp Leu Pro Phe His Ile Phe Phe Leu Leu Pro Tyr  
 15 260 265 270  
 Ile Asn Pro Asp Leu Tyr Leu Lys Lys Phe Ile Gln Gln Val Tyr Leu  
 275 280 285  
 20 Ala Ile Met Trp Leu Ala Met Ser Ser Thr Met Tyr Asn Pro Ile Ile  
 290 295 300  
 Tyr Cys Cys Leu Asn Asp Arg Phe Arg Leu Gly Phe Lys His Ala Phe  
 305 310 315 320  
 25 Arg Cys Cys Pro Phe Ile Ser Ala Gly Asp Tyr Glu Gly Leu Glu Met  
 325 330 335  
 Lys Ser Thr Arg Tyr Leu Gln Thr Gln Gly Ser Val Tyr Lys Val Ser  
 30 340 345 350  
 Arg Leu Glu Thr Thr Ile Ser Thr Val Val Gly Ala His Glu Glu Glu  
 355 360 365  
 35 Pro Glu Asp Gly Pro Lys Ala Thr Pro Ser Ser Leu Asp Leu Thr Ser  
 370 375 380  
 Asn Cys Ser Ser Arg Ser Asp Ser Lys Thr Met Thr Glu Ser Phe Ser  
 385 390 395 400  
 40 Phe Ser Ser Asn Val Leu Ser  
 405

## (2) INFORMATION FOR SEQ ID NO:30:

- 45 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1679 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 50 (D) TOPOLOGY: linear  
 55

## (ii) MOLECULE TYPE:

5

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GAAAAAGCCT TCCACCCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GGCGCCACGA 60  
 CAGGACTCTG CTGCAGAGGG GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG 120  
 AAATGGATAA CGTCTCCCG GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT 180  
 CGGAACCCAA TCAGTTCGTG CAACCAGCCT GGCAAATTGT CCTTTGGGCA GCTGCCTACA 240  
 CGGTCATTGT GGTGACCTCT GTGGTGGGCA ACGTGGTAGT GATGTGGATC ATCTTAGCCC 300  
 ACAAAGAAT GAGGACAGTG ACGAACTATT TTCTGGTGAA CCTGGCCTTC GCGGAGGCCT 360  
 CCATGGCTGC ATTCAATACA GTGGTGAAC TACCTATGC TGCCACAAC GAATGGTACT 420  
 ACGGCCTGTT CTACTGCAAG TTCCACAACT TCTTCCCAT CGCCGCTGTC TTCGCCAGTA 480  
 TCTACTCCAT GACGGCTGTG GCCTTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC 540  
 CCCGGCTGTC AGCCACAGCC ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC 600  
 TGCTGGCCTT CCCCAGGGC TACTACTCAA CCACAGAGAC CATGCCCAGC AGAGTCGTGT 660  
 GCATGATCGA ATGGCCAGAG CATCCGAACA AGATTTATGA GAAAGTGAC CACATCTGTG 720  
 TGACTGTGCT GATCTACTTC CTCCCCCTGC TGGTGATTGG CTATGCATAC ACCGTAGTGG 780  
 GAATCACACT ATGGGECAGT GAGATCCCCG GGGATCTCTC TGACCGCTAC CACGAGCAAG 840  
 TCTGTGCCAA GCGCAAGGTG GTCAAAATGA TGATTGTCTG GTGTGCACC TTCGCCATCT 900  
 GTGGGTGCC CTTCACATC TTCTTCTCTC TGCCCTACAT CAACCCAGAT CTCTACCTGA 960  
 AGAAGTTTAT CCAGCAGGTC TACCTGCCA TCATGTGGCT GGCCATGAGC TCCACCATGT 1020  
 ACAACCCCAT CATCTACTGC TGGCTCAATG ACAGGTTCGG TCTGGGCTTC AAGCATGCCT 1080  
 TCCGGTGTG CCCCITCATC AGCGCGGGCG ACTATGAGGG GCTGGAAATG AAATCCACCC 1140  
 GGTATCTCCA GACCCAGGGC AGTGTGTACA AAGTCAGCCG CCTGGAGACC ACCATCTCCA 1200  
 CAGTGGTGGG GGCCACGAG GAGGAGCCAG AGGACGGCCC CAAGGCCACA CCCTCGTCCC 1260  
 TGGACCTGAC CTCCAACCTGC TCTTACGAA GTGACTCAA GACCATGACA GAGAGCTTCA 1320

50

55



EP 0 510 878 A1

	GCTTCTCCTC CAATGTGCTC TCCTAGGCCA CAGGGCCTTT GGCAGGTGCA GCCCCCACTG	1380
5	CCTTTGACCT GCCTCCCTTC ATGCATGGAA ATTCCCTTCA TCTGGAACCA TCAGAAACAC	1440
	CCTCACACTG GGACTIONGCAA AAAGGGTCAG TATGGGTTAG GGAAAACATT CCATCCTTGA	1500
	GTCAAAAAAT CTCAATTCTT CCCTATCTTT GCCACCCTCA TGCTGTGTGA CTCAAACCAA	1560
10	ATCACTGAAC TTTGCTGAGC CTGTAAAATA AAAGGTCGGA CCAGCTTTTC CAAAAGCCC	1620
	ATTCATTCCA TTCTGGAAGT GACTTTGGCT GCATGCGAGT GCTCATTCA GGATGAATT	1679

15

20

25

30

35

40

45

50

55

## Claims

1. A human neurokinin-I receptor, the receptor being substantially free of other-human receptor proteins.
- 5 2. A protein corresponding to the amino acid sequence of human neurokinin-I receptor, the protein consisting of 407 amino acids.
3. A protein corresponding to the amino acid sequence which is:

10	10	20
	Met Asp Asn Val Leu Pro Val Asp Ser Asp Leu Ser Pro Asn Ile Ser Thr Asn Thr Ser	
	30	40
15	Glu Pro Asn Gln Phe Val Gln Pro Ala Trp Gln Ile Val Leu Trp Ala Ala Ala Tyr Thr	
	50	60
	Val Ile Val Val Thr Ser Val Val Gly Asn Val Val Val Met Trp Ile Ile Leu Ala His	
	70	80
20	Lys Arg Met Arg Thr Val Thr Asn Tyr Phe Leu Val Asn Leu Ala Phe Ala Glu Ala Ser	
	90	100
	Met Ala Ala Phe Asn Thr Val Val Asn Phe Thr Tyr Ala Val His Asn Glu Trp Tyr Tyr	

25

30

35

40

45

50

55

110 120  
 Gly Leu Phe Tyr Cys Lys Phe His Asn Phe Phe Pro Ile Ala Ala Val Phe Ala Ser Ile  
 130 140  
 5 Tyr Ser Met Thr Ala Val Ala Phe Asp Arg Tyr Met Ala Ile Ile His Pro Leu Gln Pro  
 150 160  
 Arg Leu Ser Ala Thr Ala Thr Lys Val Val Ile Cys Val Ile Trp Val Leu Ala Leu Leu  
 10 170 180  
 Leu Ala Phe Pro Gln Gly Tyr Tyr Ser Thr Thr Glu Thr Met Pro Ser Arg Val Val Cys  
 190 200  
 15 Met Ile Glu Trp Pro Glu His Pro Asn Lys Ile Tyr Glu Lys Val Tyr His Ile Cys Val  
 210 220  
 Thr Val Leu Ile Tyr Phe Leu Pro Leu Leu Val Ile Gly Tyr Ala Tyr Thr Val Val Gly  
 230 240  
 20 Ile Thr Leu Trp Ala Ser Glu Ile Pro Gly Asp Ser Ser Asp Arg Tyr His Glu Gln Val  
 250 260  
 Ser Ala Lys Arg Lys Val Val Lys Met Met Ile Val Val Val Cys Thr Phe Ala Ile Cys  
 25 270 280  
 Trp Leu Pro Phe His Ile Phe Phe Leu Leu Pro Tyr Ile Asn Pro Asp Leu Tyr Leu Lys  
 290 300  
 30 Lys Phe Ile Gln Gln Val Tyr Leu Ala Ile Met Trp Leu Ala Met Ser Ser Thr Met Tyr  
 310 320  
 Asn Pro Ile Ile Tyr Cys Cys Leu Asn Asp Arg Phe Arg Leu Gly Phe Lys His Ala Phe  
 330 340  
 35 Arg Cys Cys Pro Phe Ile Ser Ala Gly Asp Tyr Glu Gly Leu Glu Met Lys Ser Thr Arg  
 350 360  
 Tyr Leu Gln Thr Gln Gly Ser Val Tyr Lys Val Ser Arg Leu Glu Thr Thr Ile Ser Thr  
 370 380  
 40 Val Val Gly Ala His Glu Glu Glu Pro Glu Asp Gly Pro Lys Ala Thr Pro Ser Ser Leu  
 390 400  
 Asp Leu Thr Ser Asn Cys Ser Ser Arg Ser Asp Ser Lys Thr Met Thr Glu Ser Phe Ser  
 45 407  
 Phe Ser Ser Asn Val Leu Ser

4. A DNA sequence, encoding human neurokin-I receptor, the sequence being free of other human DNA sequences.
5. A DNA sequence comprising the sequence which is:

130 140  
 ATGGATAA CGTCCTCCCG

5 150 160 170 180 190 200 210  
 GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT CGGAACCCAA TCAGTTCGTG CAACCAGCCT

220 230 240 250 260 270 280  
 GGCAAATTGT CCTTTGGGCA GCTGCCTACA CGGTCAATTGT GGTGACCTCT GTGGTGGGCA ACGTGGTAGT

10 290 300 310 320 330 340 350  
 GATGTGGATC ATCTTAGCCC ACAAAGAAT GAGGACAGTG ACGAACTATT TTCTGGTGAA CCTGGCCTTC

360 370 380 390 400 410 420  
 GCGGAGGCCT CCATGGCTGC ATTCAATACA GTGGTGAAGT TCACCTATGC TGTCCACAAC GAATGGTACT

15 430 440 450 460 470 480 490  
 ACGGCCTGTT CTACTGCAAG TTCCACAAGT TCTTCCCAT CGCCGCTGTC TTCGCCAGTA TCTACTCCAT

500 510 520 530 540 550 560  
 GACGGCTGTG GCCTTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC CCCGGCTGTC AGCCACAGCC

20 570 580 590 600 610 620 630  
 ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC TGCTGGCCTT CCCCCAGGGC TACTACTCAA

25 640 650 660 670 680 690 700  
 CCACAGAGAC CATGCCCAGC AGAGTCGTGT GCATGATCGA ATGGCCAGAG CATCCGAACA AGATTATGA

710 720 730 740 750 760 770  
 GAAAGTGAC CACATCTGTG TGAATGTGCT GATCTACTTC CTCCCCCTGC TGGTGATTGG CTATGCATAC

30 780 790 800 810 820 830 840  
 ACCGTAGTGG GAATCACACT ATGGGCCAGT GAGATCCCCG GGGACTCCTC TGACCGCTAC CACGAGCAAG

850 860 870 880 890 900 910  
 TCTCTGCCAA GCGCAAGGTG GTCAAATGA TGATTGTCGT GGTGTGCACC TTCGCCATCT GCTGGCTGCC

35 920 930 940 950 960 970 980  
 CTTCCACATC TTCTTCTCC TGCCCTACAT CAACCCAGAT CTCTACCTGA AGAAGTTTAT CCAGCAGGTC

990 1000 1010 1020 1030 1040 1050  
 TACCTGGCCA TCATGTGGCT GGCCATGAGC TCCACCATGT ACAACCCCAT CATCTACTGC TGCCTCAATG

40 1060 1070 1080 1090 1100 1110 1120  
 ACAGGTTCCG TCTGGGCTTC AAGCATGCCT TCCGGTGCTG CCCCTTCATC AGCGCCGGCG ACTATGAGGG

45 1130 1140 1150 1160 1170 1180 1190  
 GCTGGAAATG AAATCCACCC GGTATCTCCA GACCCAGGGC AGTGTGTACA AAGTCAGCCG CCTGGAGACC

1200 1210 1220 1230 1240 1250 1260  
 ACCATCTCCA CAGTGGTGGG GGCCACGAG GAGGAGCCAG AGGACGGCCC CAAGGCCACA CCCTCGTCCC

50 1270 1280 1290 1300 1310 1320 1330  
 TGGACCTGAC CTCCAAGTGC TCTTACGAA GTGACTCCAA GACCATGACA GAGAGCTTCA GCTTCTCCTC

1340 1346  
 CAATGTGCTC TCCTAG

55

or a degenerate variation thereof.

6. The DNA sequence of Claim 5 further comprising the sequence which is:

```

      10      20      30      40      50      60      70
5  GAAAAAGCCT TCCACCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GGCGCCACGA CAGGACTCTG

      80      90      100      110      120 122
    CTGCAGAGGG GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG AA

```

10 or a degenerate variation thereof.

7. The DNA sequence of Claim 6 further comprising the sequence which is:

```

      1350      1360      1370      1380      1390      1400
15  GCCA CAGGGCCTTT GGCAGGTGCA GCCCCCACTG CCTTTGACCT GCCTCCCTTC

      1410      1420      1430      1440      1450      1460      1470
    ATGCATGGAA ATTCCCTTCA TCTGGAACCA TCAGAAACAC CCTCACACTG GGACTTGCAA AAAGGGTCAG

20      1480      1490      1500      1510      1520      1530      1540
    TATGGGTTAG GGAACACATT CCATCCTTGA GTCAAAAAAT CTCAATTCTT CCCTATCTTT GCCACCCTCA

      1550      1560      1570      1580      1590      1600      1610
    TGCTGTGTGA CTCAAACCAA ATCACTGAAC TTTGCTGAGC CTGTAAATA AAAGGTCGGA CCAGCTTTTC

25      1620      1630      1640      1650      1660      1670      1679
    CCAAAGCCC ATTCATTCCA TTCTGGAAGT GACTTTGGCT GCATGCGAGT GCTCATTTC A GGATGAATT

```

or a degenerate variation thereof.

- 30 8. A plasmid which comprises:  
 (a) a mammalian expression vector, and  
 (b) a base sequence encoding human neurokinin-I receptor protein.
- 35 9. A method of using a Chinese hamster ovarian cell line (CHO), the cell line transplanted with a plasmid, which plasmid comprises:  
 (a) a mammalian expression vector, and  
 (b) a base sequence encoding human neurokinin-I receptor protein,  
 the method which comprises:  
 (1) expressing human neurokinin-I receptor in the CHO cells;  
 (2) adding a test sample to a solution containing <sup>125</sup>I-substance P and the CHO cells;  
 (3) incubating the products of Step 2, wherein the incubation is effective for competitive binding of the <sup>125</sup>I-substance P and the test sample to the human neurokinin-I receptor;  
 (4) separating the <sup>125</sup>I-substance P which is bound to the human neurokinin-I receptor from the <sup>125</sup>I-substance P which is not bound;  
 (4) measuring the amount of the <sup>125</sup>I-substance P which is bound to the human neurokinin-I receptor.
- 40 10. A method of using a Chinese hamster ovarian cell line (CHO), the line transplanted with a plasmid, which plasmid comprises:  
 (a) a mammalian expression vector, and  
 (b) a base sequence encoding human neurokinin-I receptor protein,  
 the method which comprises:  
 (1) expressing human neurokinin-I receptor in the CHO cells;  
 (2) equilibrating the product of Step (1) with <sup>3</sup>H-myoinositol;  
 (3) washing the product of Step (2);  
 (4) incubating the product of Step (3) with a test sample in the presence of aqueous LiCl, resulting in the production of <sup>3</sup>H-inositol monophosphate;  
 (5) measuring the <sup>3</sup>H-inositol monophosphate.
- 50
- 55

FIGURE 1

10	20
Met Asp Asn Val Leu Pro Val Asp Ser Asp Leu Ser Pro Asn Ile Ser Thr Asn Thr Ser	
30	40
Glu Pro Asn Gln Phe Val Gln Pro Ala Trp Gln Ile Val Leu Trp Ala Ala Ala Tyr Thr	
50	60
Val Ile Val Val Thr Ser Val Val Gly Asn Val Val Val Met Trp Ile Ile Leu Ala His	
70	80
Lys Arg Met Arg Thr Val Thr Asn Tyr Phe Leu Val Asn Leu Ala Phe Ala Glu Ala Ser	
90	100
Met Ala Ala Phe Asn Thr Val Val Asn Phe Thr Tyr Ala Val His Asn Glu Trp Tyr Tyr	
110	120
Gly Leu Phe Tyr Cys Lys Phe His Asn Phe Phe Pro Ile Ala Ala Val Phe Ala Ser Ile	
130	140
Tyr Ser Met Thr Ala Val Ala Phe Asp Arg Tyr Met Ala Ile Ile His Pro Leu Gln Pro	
150	160
Arg Leu Ser Ala Thr Ala Thr Lys Val Val Ile Cys Val Ile Trp Val Leu Ala Leu Leu	
170	180
Leu Ala Phe Pro Gln Gly Tyr Tyr Ser Thr Thr Glu Thr Met Pro Ser Arg Val Val Cys	
190	200
Met Ile Glu Trp Pro Glu His Pro Asn Lys Ile Tyr Glu Lys Val Tyr His Ile Cys Val	
210	220
Thr Val Leu Ile Tyr Phe Leu Pro Leu Leu Val Ile Gly Tyr Ala Tyr Thr Val Val Gly	
230	240
Ile Thr Leu Trp Ala Ser Glu Ile Pro Gly Asp Ser Ser Asp Arg Tyr His Glu Gln Val	
250	260
Ser Ala Lys Arg Lys Val Val Lys Met Met Ile Val Val Val Cys Thr Phe Ala Ile Cys	

FIGURE 1 (continued)

270	280
Trp Leu Pro Phe His Ile Phe Phe Leu Leu Pro Tyr Ile Asn Pro Asp Leu Tyr Leu Lys	
290	300
Lys Phe Ile Gln Gln Val Tyr Leu Ala Ile Met Trp Leu Ala Met Ser Ser Thr Met Tyr	
310	320
Asn Pro Ile Ile Tyr Cys Cys Leu Asn Asp Arg Phe Arg Leu Gly Phe Lys His Ala Phe	
330	340
Arg Cys Cys Pro Phe Ile Ser Ala Gly Asp Tyr Glu Gly Leu Glu Met Lys Ser Thr Arg	
350	360
Tyr Leu Gln Thr Gln Gly Ser Val Tyr Lys Val Ser Arg Leu Glu Thr Thr Ile Ser Thr	
370	380
Val Val Gly Ala His Glu Glu Glu Pro Glu Asp Gly Pro Lys Ala Thr Pro Ser Ser Leu	
390	400
Asp Leu Thr Ser Asn Cys Ser Ser Arg Ser Asp Ser Lys Thr Met Thr Glu Ser Phe Ser	
407	
Phe Ser Ser Asn Val Leu Ser	

FIGURE 2

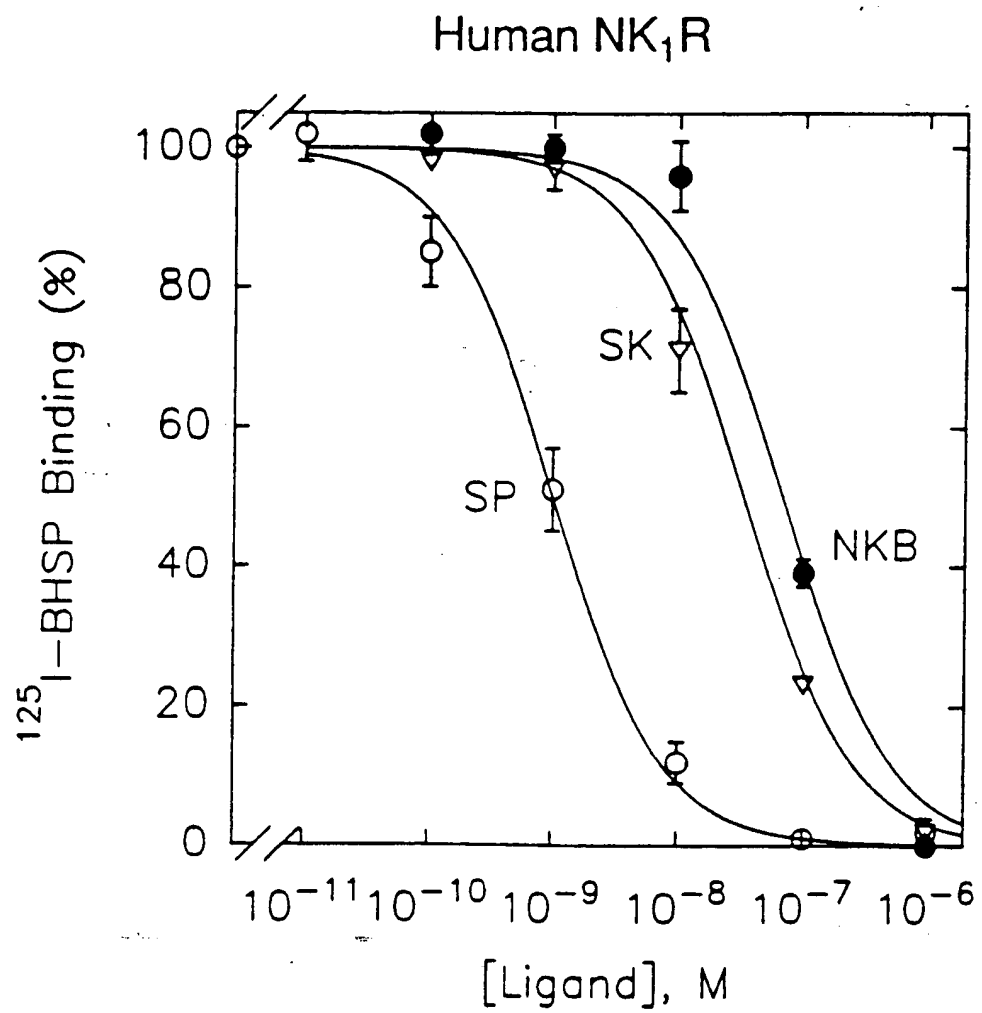
10	20	30	40	50	60	70
GAAAAAGCCT TCCACCCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GCGCCACGA CAGGACTCTG						
80	90	100	110	120	130	140
CTGCAGAGGC GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG AAATGGATAA CGTCCTCCCC						
150	160	170	180	190	200	210
GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT CGGAACCCAA TCAGTTCGTC CAACCAGCCT						
220	230	240	250	260	270	280
GGCAAATTGT CCTTTGGGCA GCTGCCTACA CGGTCATTGT GGTGACCTCT GTGGTGGGCA ACGTGGTAGT						
290	300	310	320	330	340	350
GATGTGGATC ATCTTACCCC ACAAAGAAT GAGGACAGTG ACCAACTATT TTCTGCTGAA CCTGGCCTTC						
360	370	380	390	400	410	420
GCGGAGGCCT CCATGGCTGC ATTCAATACA GTGGTGAAC TACCTATGC TGTCCACAAC GAATGGTACT						
430	440	450	460	470	480	490
ACGGCCTGTT CTA CTGCAAG TTCCACAAC TCTTCCCCAT CCGCGCTGTC TTGCGCAGTA TCTACTCCAT						
500	510	520	530	540	550	560
GACGGCTGTG GCCTTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC CCGCGCTGTC AGCCACAGCC						
570	580	590	600	610	620	630
ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC TGCTGGCCTT CCCCAGGGC TACTACTCAA						
640	650	660	670	680	690	700
CCACAGAGAC CATGCCCAGC AGAGTCGTGT GCATGATCGA ATGCCCAGAC CATCCGAACA AGATTATGA						
710	720	730	740	750	760	770
GAAAGTGTAC CACATCTGTC TGA CTGTGCT GATCTACTTC CTCCCCCTGC TGGTGATTGG CTATGCATAC						
780	790	800	810	820	830	840
ACCGTAGTGG GAATCACACT ATGGGCCAGT GAGATCCCCG GGGACTCCTC TGACCGCTAC CACGAGCAAG						
850	860	870	880	890	900	910
TCTCTGCCAA GCGCAAGGTG GTCAAAATGA TGATTGTCGT GGTGTGCACC TTCGCCATCT GCTGGCTGCC						



FIGURE 2 (continued)

920	930	940	950	960	970	980
CTTCCACATC	TTCTTCCTCC	TGCCCTACAT	CAACCCAGAT	CTCTACCTCA	AGAAGTTTAT	CCAGCAGGTC
990	1000	1010	1020	1030	1040	1050
TACCTGGCCA	TCATGTGGCT	GGCCATGAGC	TCCACCATGT	ACAACCCCAT	CATCTACTGC	TGCCTCAATG
1060	1070	1080	1090	1100	1110	1120
ACAGGTTCCG	TCTGGGCTTC	AAGCATGCCT	TCCGGTGCTG	CCCCTTCATC	AGCGCCGGCG	ACTATGAGGG
1130	1140	1150	1160	1170	1180	1190
GCTGGAAATC	AAATCCACCC	GGTATCTCCA	GACCCAGGGC	AGTGTGTACA	AAGTCAGCCC	CCTGGAGACC
1200	1210	1220	1230	1240	1250	1260
ACCATCTCCA	CAGTGGTGGG	GGCCCACGAG	GAGGAGCCAG	AGGACGGCCC	CAAGGCCACA	CCCTCGTCCC
1270	1280	1290	1300	1310	1320	1330
TGGACCTGAC	CTCCAAGTGC	TCTTCACGAA	GTGACTCCAA	GACCATGACA	GAGAGCTTCA	GCTTCTCCTC
1340	1350	1360	1370	1380	1390	1400
CAATGTGCTC	TCCTAGGCCA	CAGGGCCTTT	GGCAGGTGCA	GGCCCCACTG	CCTTTGACCT	GCCTCCCTTC
1410	1420	1430	1440	1450	1460	1470
ATGCATGGAA	ATTCCCTTCA	TCTGGAACCA	TCAGAAACAC	CCTCACACTG	GGACTTGCAA	AAAGGGTCAG
1480	1490	1500	1510	1520	1530	1540
TATGGGTTAG	GGAAAACATT	CGATCCTTGA	GTCAAAAAAT	CTCAATTCTT	CCCTATCTTT	GCCACCCCTCA
1550	1560	1570	1580	1590	1600	1610
TGCTGTCTGA	CTCAAACCAA	ATCACTGAAC	TTTGCTGAGC	CTGTAAAATA	AAAGCTCCGA	CCAGCTTTTC
1620	1630	1640	1650	1660	1670	1679
CCAAAAGCCC	ATTCATTCCA	TTCTGGAAGT	GACTTTGGCT	GCATGCCAGT	GCTCATTTC	GGATGAATT

FIGURE 3





European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number

EP 92 30 3457  
Page 1

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P, X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 180, no. 2, 31 October 1991, DULUTH, MINNESOTA US pages 1110 - 1117; Hopkins, B. et al.: 'Isolation and characterization of the human lung NK-1 receptor cDNA.' * the whole document *	1-10	C12N15/12 C07K13/00 G01N33/58 G01N33/68
P, X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 179, no. 3, 30 September 1991, DULUTH, MINNESOTA US pages 1231 - 1240; Takeda Y; Chou KB; Takeda J; Sachals BS; Krause JE: 'Molecular cloning, structural characterization and functional expression of the human substance P receptor.' * the whole document *	1-10	
P, X	BIOCHEMISTRY. vol. 30, no. 44, 5 November 1991, EASTON, PA US pages 10640 - 10646; Garard NP; Garraway LA; Eddy RL Jr; Shows TB; Iijima H; Paquet JL; Gerard C: 'Human substance P receptor (NK-1): organization of the gene, chromosome localization, and functional expression of cDNA clones.' * the whole document *	1-10	TECHNICAL FIELDS SEARCHED (Int. Cl.5)  C07K C12N
X	SCIENCE. vol. 247, 23 February 1990, LANCASTER, PA US pages 958 - 952; Hershey AD; Krause JE: 'Molecular characterization of a functional cDNA encoding the rat substance P receptor.' * the whole document *	1-10	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 16 JULY 1992	Examiner NAUCHE S, A.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document	

EPO FORM 1501 (12.92) (P0401)



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number

EP 92 30 3457

Page 2

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 30, 25 October 1990, BALTIMORE US pages 17649 - 17652; Yokota Y; Sasai Y; Tanaka K; Fujiwara T; Tsuchida K; Shigemoto R; Kakizuka A; Ohkubo H; Nakanishi S: 'Molecular characterization of a functional cDNA for rat substance P receptor.' * the whole document *	1-10	
P,X	PATENT ABSTRACTS OF JAPAN vol. 015, no. 341 29 August 1991 & JP-A-30 133 998 ( MITSUBISHI KASEI CORP ) 7 June 1991 * abstract *	1-8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 16 JULY 1992	Examiner NAUCHE S.A.
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone  Y : particularly relevant if combined with another document of the same category  A : technological background  O : non-written disclosure  P : intermediate document</p> <p>T : theory or principle underlying the invention  E : earlier patent document, but published on, or after the filing date  D : document cited in the application  L : document cited for other reasons  &amp; : member of the same patent family, corresponding document</p>			

EPO FORM 1503 01.92 (P0401)